a method of modifying a terminal heptose of a lipopolysaccharide (LPS) or lipooligosaccharide (LOS) core structure of a gram negative bacterial species containing an *rfe* (UDP-GlcNAc:Undecaprenol GlcNAc-a phosphate transferase) comprising regulating the *rfe* with an *lsgG* gene from *Haemophilus influenzae* in order to catalyze transferring N-acetyl glucosamine onto the terminal heptose. Support for this claim can be found, for example, at page 7, lines 5-21.

The recited *rfe* adds an acceptor molecule to the heptose molecule to synthesize an oligosaccharide. The DNA sequence encoding *rfe* may be part of the production cell's genome (*see*, specification at page 4 lines 29-30). Alternatively, the DNA sequence encoding *rfe* may be from *Haemophilus influenzae* (*see*, specification at page 4, line 29, through page 5, line 1; original claims 8 and 9). Support for the *lsg* gene is found in the specification, for example, at page 5, lines 2-6. Support for the core lipid structure containing a terminal heptose is found, for example, in original claim 1.

Support for "*Haemophilus influenzae*-specific LOS epitopes in *E. coli*" can be found, for example, at page 7, line 21 of the specification. A *Haemophilus influenzae*-specific LOS is a LOS that is substantially identical to that of *Haemophilus influenzae* (see, page 9 and 21 of specification) or is "essentially identical to that of *Haemophilus influenzae*" (see, page 21, line 4). Example 5 extensively describes the comparison of the structural identity of LOS naturally generated by *Haemophilus influenzae* and that generated in an *E. coli* host.

Drawings

The objections to the drawings are noted. Corrected formal drawings will be filed upon receipt of a Notice of Allowance.

§112 Rejection of the Claims

A. Written Description

Claims 1, 5-9, 11-12, 14, and 16-17 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to convey to one skilled in the art that the inventors at the time the application was filed, had possession of the claimed invention. The Examiner states that the claims comprise a vast diverse

genus of microorganisms wherein the lipooligosaccharides have different lipid core structures, thereby requiring enzymes with different substrate specificity corresponding to different lipid core structures.

It should be noted that claims 1, 16, and 17 have been canceled. Also, independent claim 11 has been amended (and claims 18-21 newly added) to expedite prosecution of the remaining claims in the present application. Insofar as the rejection is applied to the present claims, Applicants traverse the rejection.

Under the new "Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶1 Written Description Requirement," the written description requirement for a claimed genus may be satisfied by "disclosure of relevant, identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus." Fed. Reg. 66:1099-1111, 1106, (January 1, 2001).

The present claims recite a definite group of microorganisms, specifically those that have three structural features. First, the microorganisms must have core lipid structures that contain a terminal heptose molecule. Second, they must have isolated DNA sequences that encode *rfe* (UDP-GlcNAc:Undecaprenol GlcNAc-1 phosphate transferase). Third, they must have an isolated DNA sequence encoding a liposaccharide-synthesis gene (*lsg*) from *Haemophilus influenzae*. The pending claims further recite a function associated with these structural elements, namely, the *rfe* adds an acceptor molecule to the heptose molecule to synthesize a complex carbohydrate or oligosaccharide. Thus, the specification teaches common identifying characteristics of the bacteria that properly produce the desired complex carbohydrates or oligosaccharides, and therefore teaches the requisite combination of structural and functional characteristics as set for in the Written Description Guidelines.

Applicants therefore request that the rejection under 35 U.S.C. § 112, first paragraph (written description) be withdrawn.

B. Enablement

Claims 1, 5-9, 11-12, 14, and 16-17 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The claims have been amended. Insofar as the rejection is applied to the currently pending claims, it is hereby traversed.

To be enabling, the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation. *Genentech Inc. v. Novo Nordisk A/S*, 108 F.3d 1361, 42 U.S.P.Q.2d (BNA) 1001, 1004 (Fed. Cir. 1997). The scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art. *Id.* Whether making or using the invention would have required undue experimentation, and thus whether the disclosure is enabling, is a matter of degree. *PPG Industries Inc. v. Guardian Industries Corp.*, 156 F.3d 1351, 37 U.S.P.Q.2d (BNA) 1618, 1623 (Fed. Cir. 1996). The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation must not be unduly extensive. *Id.*

Applicants assert that the present patent specification teaches one skilled in the art how to make and use the full scope of the claimed invention without undue experimentation. The scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art. As described above, the pending claims recite a process for the production of an LOS or LPS. The process involves growing bacteria that contain a core lipid structure containing a terminal heptose, an isolated DNA sequence encoding *rfe*, and an isolated DNA sequence encoding *lsg* from *Haemophilus influenzae*, and recovering the oligosaccharide or complex carbohydrate from the culture medium. The recited *rfe* adds an acceptor molecule to the heptose molecule to synthesize an oligosaccharide.

The Examiner appears to be taking the view that the specification is not enabled because all the possible types of bacteria have not been tested. This, however, is not the legal standard for enablement. Some experimentation is permitted, and still have an application be enabled. The amount of experimentation simply cannot be undue in view of teaching of the specification. The factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the Federal Circuit in *In re Wands*, 858 F.2d 731, 8

U.S.P.Q.2d (BNA) 1400, 1404 (Fed. Cir. 1988). They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

The specification provides a significant amount of direction and guidance (factor 2). The specification extensively discusses in the working examples how to identify and characterize the LOS or LPS made by the bacteria using the claimed invention. Moreover, Applicants have performed further studies to provide additional evidence that the *Haemophilus influenzae lsg* locus could be used to produce chimeric LPS structure in Gram-negative bacteria other than *E. coli*. *See*, Declaration of Dr. Apicella, ¶ 2. Applicants transformed the *Salmonella minnesota* Ra mutant with the plasmid pGEM and pGEMLOS-4. *S. Minnesota* Ra contains the entire core region of this LPS and lacks any O-antigen structures. The plasmid pGEM does not contain any genetic insertion in its multicloning site while pGEMLOS-4 contains the complete 7.2 kb *lsg* in the multicloning site. Colonies of *S. minnesota* containing pGEMLOS-4 expressed a terminal epitope present on all *H. influenzae* lipooligosaccharide while those of *S. minnesota* with the plasmid alone did not express this epitope. These studies demonstrate that Gram negative bacteria other than *E. coli* can be used to make complex carbohydrates with the *lsg* locus.

The skill of those in the art (factor 6) is quite high in the field of molecular biology, as evidenced by the level of sophistication of the experiments set forth in the specification. Concerning the nature of the invention (factor 4) and the state of the prior art (factor 5), prior to the present invention, the control of *rfe* by *H. influenzae lsg* was not known. The present specification describes a novel method for constructing arrays of chimeric oligosaccharides using the LPS biosynthetic machinery in bacteria. This was an unexpected finding. The details of how this method actually works were first described in the present specification. Only after the inventors made this discovery could anyone teach how complex carbohydrates could be made using this general strategy. The breadth of the amended claims (factor 8) is commensurate with this discovery.

Regarding the quantity of experimentation necessary (factor 1) and the predictability or unpredictability of the art (factor 7), mathematically a large number of bacterial strains could be

generated and screened. With respect to "undue experimentation," the fact that the outcome of a synthesis/screening program is unpredictable is precisely why a screening program is carried out. One cannot reasonably contend that a screening program to locate bacteria synthesizing the desired complex carbohydrate or oligosaccharide would not be carried out by the art worker because the results cannot be fully predicted in advance. In fact, the Federal Circuit explicitly recognized that a need to carry out extensive synthesis and screening programs to locate bioactive molecules does not constitute undue experimentation. *In re Wands*, at 1406-1407. In *Wands* the court held that a process of immunizing animals, fusing lymphocytes from the immunized animals with myeloma cells to make hybridomas, cloning the hybridomas, and screening the antibodies produced by the hybridomas for the desired characteristics did not require undue experimentation. The Court stated:

> The nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics. Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody.

Likewise, practitioners having skill in the art related to the present application, given the teachings of the present specification, would be well-equipped to prepare bacterial species using the method of the present invention that produce the desired biomolecules (LOS or LPS). Thus, the fact that a claim may encompass a large number of bacterial species is not dispositive of the enablement issue. This is particularly true in an art area in which the level of skill is very high.

Thus, considering the *Wands* factors and the Declaration of Dr. Apicella, it would not require undue experimentation to obtain additional bacterial species commensurate in scope with the pending claims. Applicants therefore assert that the specification fully enables one skilled in the art to use the method of the present invention. The first paragraph of 35 U.S.C. §112 requires no more than a disclosure sufficient to enable one skilled in the art to carry out the invention commensurate with the scope of the claims, and this requirement has been met. It is respectfully submitted that the pending claims conform with 35 U.S.C. §112, first paragraph. Therefore, Applicant requests that the Examiner withdraw the 35 U.S.C. §112, first paragraph (enablement) rejection.

C. Indefiniteness

Claims 1, 5-9, 11-12, 14, and 16-17 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

The examiner indicated that the claims do not clearly state whether the core lipid structure and the enzyme catalyzing the addition of the acceptor molecules are inherently present in the production cell, and that more clearly defined method steps will overcome this rejection. The claims have been amended to clarify the method steps.

The examiner indicated that the term "glycotransferase" is unclear. This term has been deleted from the claims.

The examiner indicated that the term "functional" is redundant. Claim 8 has been amended to delete this term. Claim 9 has been cancelled.

The examiner indicated that it was unclear if the isolated DNA sequence is encoding a glycosyltransferase or another enzyme. Claim 12 has been amended to clarify that the DNA encodes an *rfe* sequence. Claim 14 has been cancelled.

It is respectfully submitted that the pending claims conform with 35 U.S.C. §112, first paragraph. Therefore, Applicant requests that the Examiner withdraw the 35 U.S.C. §112, first paragraph (indefiniteness) rejection.

§102 Rejections of the Claims

Kwaik *et al.* (*Molecular Microbiology* 5(10), 2475-2480)

Claims 1, 5-8, 11-12, and 16-17 were rejected under 35 USC § 102(b) as being anticipated by Kwaik *et al.* (*Molecular Microbiology* 5(10), 2475-2480).

Rejection for anticipation required, as the first step in the inquiry, that all the elements of the claimed invention be described in a single reference. *In re Spada*, 15 U.S.P.Q.2d 1655, 1657 (Fed. Cir. 1990). Absence from the reference of any claimed element negates anticipation. *Rowe v. Dror*, 42 U.S.P.Q.2d 1550, 1553 (Fed. Cir. 1997). Further, an anticipating reference must describe the patented subject matter with sufficient clarity and detail to establish that the subject matter existed and that its existence was recognized by persons of ordinary skill in the field of

the invention. *ATD Corp. v. Lydall Inc.*, 48 U.S.P.Q.2d 1321, 1328 (Fed. Cir. 1998) (emphasis added).

Kwaik teaches that a *Haemophilus influenzae* 7.2 kb PstI-BamHI restriction fragment contains a cluster of at least three genetic loci whose products act sequentially in LOS synthesis. Kwaik at page 2475. They also teach that when these genes are inserted into *E. coli*, a 5.5 K LPS species is made that contains a 2-keto-deoxyoctulosonic acid (KDO) epitope as evidence by its ability to bind to a KDO-specific monoclonal antibody. *Id.* The data confirmed that the cloned genes functioned in *Haemophilus influenzae* LOS biosynthesis. The genes were responsible for the sequential assembly of three oligosaccharide components on *E. coli* LPS. Kwaik at page 2477. As stated repeatedly in the paper, the authors believe that *E. coli* LPS was being formed rather than *Haemophilus influenzae*-specific LOS. They stated that the gene products of the *lsg-1* locus may recognize an *E. coli* LPS acceptor residue, and modification of *E. coli* LPS by *lsg-1* may be required for the functioning of other gene products encoded by the cloned fragment. Kwaik *et al.* at p. 2477. Alternatively, the genes contained in the cloned fragment may be part of a complex operon that requires the presence of the *lsg-1* promoter for transcription, or *lsg-1* may encode a regulatory element. *Id.*; *see also*, specification at page 9, line 12. They did not recognize, however, that a *Haemophilus influenzae*-specific LOS was formed in the *E. coli* cell.

Further, Kwaik *et al.* do not discuss *lsgG* at all, and certainly do not discuss that it could be used to regulate the *rfe* with an *lsgG* gene from *Haemophilus influenzae* in order to catalyze transferring N-acetyl glucosamine onto the terminal heptose. The specification teaches that the assembly of this chimeric structure in *E. coli* was controlled by the unique interaction of *E. coli rfe* and *H. influenzae lsg.* In this interaction the regulator, *lsg*, increases the expression of *rfe*. This results in the deposition of an *N*-acetylglucosamine on the terminal heptose in the *E. coli* core region. This is a novel activity for *rfe* which was previously shown to be involved in O-antigen biosynthesis. The *N*-acetylglucosamine can act as an acceptor for other glycosyltransferases to allow assembly of a variety of oligosaccharides on the *E. coli* core structure. Therefore, the *H. influenzae lsg* control of the *rfe*, such as from *E. coli*, results in a *N*-acetylglucosamine modified core which allows the enzymatic assembly of other carbohydrates.

Since the present invention was not recognized by persons of ordinary skill in the field of the invention at the time of the Kwaik *et al.* reference, it is not anticipated by Kwaik *et al.*

Gotschlich *et al.* (U.S. 5,705,367)

Claims 1, 5, 7, 9, 11, 14 and 16-17 were rejected under 35 USC § 102(b) as being anticipated by Gotschlich *et al.* (U.S. 5,705,367).

The work by Gotschlich only describes the *Neisseria* genes encoding enzymes responsible for biosynthesis of gonococcal lipopolysaccharide terminal sugars. Further, the paper does not describe the assembly of *Haemophilus influenzae*-specific complex carbohydrates. Moreover, the paper does not mention *lsg* at all.

Thus, the present invention is patentable over the Gotschlich *et al.* reference.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-373-6961) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 6th day of November, 2001.

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Name

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Signature